New Races of *Colletotrichum lindemuthianum* in Michigan and Implications in Dry Bean Resistance Breeding

JAMES D. KELLY, LUCIA AFANADOR, and LOWELL S. CAMERON, Department of Crop and Soil Sciences, Michigan State University, East Lansing 48824

ABSTRACT

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Four isolates of *Colletotrichum lindemuthianum*, pathogenic on previously resistant dry bean (*Phaseolus vulgaris*) cultivars, were collected in 1993 in Michigan and North Dakota from seeds produced in Michigan. Characterization of the isolates on two sets of differential dry bean cultivars demonstrated that three isolates were similar and were classified as race 73. These isolates resembled the alpha-Brazil race recently reported in Ontario. The fourth isolate was unique and was classified as race 7. This isolate resembled most closely the delta race identified in Ontario in 1976. This is the first report of the occurrence of either race 7 or 73 of *C. lindemuthianum* in Michigan. Although the origin of these races is unknown, race 73 appeared to have been present in Michigan State University bean breeding lines since 1991 but was not detected until 1993, when resistant cultivars showed typical anthracnose symptoms. The presence of these races in Michigan threatens current commercial cultivars, since race 73 overcomes the *Are* gene and race 7 overcomes the *A* gene, both of which have been extensively used in the breeding program. The occurrence of these new races in North America challenges current breeding strategies of using single gene resistance to control anthracnose. Gene pyramiding using molecular markers as a disease resistance strategy is discussed, since the *A*/*Are* gene combination affords resistance to both races.

Colletotrichum lindemuthianum (Sacc. & Magnus) Lams.-Scrib., the causal agent of bean anthracnose, is a serious seedborne pathogen of common bean (*Phaseolus vulgaris* L.), particularly in cool, humid environments. Disease control measures include seed and foliar treatment with fungicides, crop rotation, certified seed production in semiarid regions, and genetic resistance. The existence of numerous physiological races of *C. lindemuthianum* reduces the usefulness of single resistance genes. Previously characterized races identified in North America include alpha, beta, gamma, delta, epsilon, and lambda in Canada (15), while only the alpha, beta, and gamma races have been reported in the United States (19). A broader array of physiological races has been reported in Europe (5,15), Latin America (1,12, 14,15), and Africa (12). Breeding for resistance in the United States has focused on the use of the *A* gene for control of the alpha race (2) and more recently on the use of the *Are* gene (11), which conditions resistance to six physiologic races (15). The *Are* gene has been used extensively in Europe (5) and Ontario (15).

In 1993, two isolates of *C. lindemuthianum* were recovered from two dry bean cultivars resistant to the common alpha race at two different locations in Michigan. A third isolate was received from North Dakota on pod tissue originating from seeds produced in Michigan in 1991. This study was undertaken to characterize these isolates of *C. lindemuthianum* based on their reaction on differential bean cultivars.

Address correspondence to first author.

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MATERIALS AND METHODS

Fungal material. Bean pods with anthracnose lesions were collected in summer 1993 from three different cultivars grown in Michigan. One sample was collected from the light red kidney cv. Isabella (8) grown on the Montcalm Research Farm, Entrican, Michigan; a second was collected from the tropical black cv. Blackhawk (6) grown on the Agronomy Farm of Michigan State University (MSU), East Lansing; and a third was recovered from a breeding line, XAN 273, introduced from the Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, and grown adjacent to Blackhawk. A fourth sample was received from North Dakota on pods of the pinto bean cv. Aztec (9) originating from seeds produced in Michigan in 1991. All diseased samples exhibited characteristic anthracnose symptoms as either rust-colored small lesions or cankerlike lesions with black rings and centers light buff in color and containing pink spore masses (13). Isolations were made from infected pods. Small pieces of infected tissue were surface-sterilized with 70% ethanol for 1 min and then with 1% NaOCl for 2 min, rinsed in sterilized distilled water, and incubated on petri dishes containing potato-dextrose agar (PDA) in complete darkness for 10 days at 24 C. All diseased pod samples from Isabella (I), Blackhawk (B), XAN 273 (X), and Aztec (A) developed fungal colonies morphologically resembling *C. lindemuthianum* (13,20). Mycelial plugs were transferred to fresh PDA for purification and incubated in complete darkness for 10 days at 24 C on PDA, bean pod agar, or bean leaf-amended agar and Mathur's agar (10) to induce sporulation. Cultures of all isolates and known races of *C. lindemuthianum* were maintained in stocks of fungus-colonized filter paper for long-term storage at −20 or were used immediately to start active cultures. Three known races of *C. lindemuthianum*—alpha (ATCC 18987), beta (ATCC 16989), and delta (ATCC 18989)—were used to compare with the unknown isolates. The ATCC 18987 culture was obtained from J. C. Tu (Agriculture Canada, Harrow, Ont.) and the ATCC 16989 and ATCC 18989 cultures were obtained from H. R. Dillard (Cornell University, Geneva, NY). Inocula of all four isolates and two characterized races, alpha and delta, were produced by growing all six isolates on Mathur's agar. All cultures were incubated for 10 days under complete darkness at 24 C. Spore suspensions were prepared by flooding plates with 5 ml of 0.01% Tween 80 in distilled water. Spores were dislodged by scraping the culture surface with a spatula. Suspensions for seedling inoculation were filtered through cheesecloth, and the concentration was adjusted to 1×10^6 spores per milliliter with a hemacytometer.

Plant material. Pinto cv. U.I. 114 was used to test pathogenicity and reisolate the four unknown isolates. Two sets of differential cultivars were used for anthracnose race characterization of the unknown isolates in comparison with the known alpha and delta races, which were used for reference purposes. The first set of eight differential cultivars (seed provided by E. Drijfhout, University of Wageningen) was proposed by Drijfhout and Davis (4), and these were used to characterize the unknown isolates (A, B, I, X). A second set of 12 differential cultivars (seed provided by M. Pastor-Corrales, CIAT) was proposed by CIAT (3), and these were used to further characterize the unknown isolates (B, I) and permit race designation. Six commercial cultivars, including those from which the isolates were obtained and cvs. Montcalm and Seafarer, also were inoculated.

Seeds of all cultivars were planted in flats containing Bacto planting mix (Michigan Peat Co., Houston, TX), and seedlings were maintained under greenhouse conditions (16-hr day length at 25 C) for 10–12 days prior to inoculation.

Inoculation and evaluation methods. Seedlings 10–12 days old (primary leaf stage) of cv. U.I. 114 were spray-inoculated separately with spore suspensions of each of the six isolates of *C. lindemuthianum*. Suspensions were applied to runoff on the stem and both surfaces of unifoliolate leaves of plants maintained in high (>95%) relative humidity for 48 hr at 22–25 C. The plants were allowed to dry and then were transferred to greenhouse benches for 7 days before disease symptoms were observed. All four unknown isolates produced typical anthracnose symptoms on leaves and stems. The pathogen was reisolated from diseased stem and leaf tissue using methods described for the initial isolation. Inoculum was prepared as before. Six to 10 greenhouse-grown seedlings of each differential cultivar (3,4) were inoculated as described and rated for disease reaction 7 days later. Disease reactions were recorded as resistant for those plants with no visible disease symptoms or only a few, very small lesions mostly on primary veins of the lower leaves. Plants with numerous enlarged lesions or with sunken cankers on the lower sides of leaves or hypocotyls were recorded as susceptible. Since the A, B, and X isolates were similar on the basis of their pathogenicity on eight differential and six commercial cultivars (Table 1), only the B isolate from this group and the I isolate were selected to inoculate the 12 CIAT differentials (Table 2). Inoculations on the two sets of differentials were repeated at least twice, and single-spore isolations of the B and I isolates were also inoculated on the CIAT differentials.

The numerical system used to characterize and distinguish different races is based on the sum of the binary values assigned to those CIAT differential cultivars on which the unknown race is pathogenic (3). This system of nomenclature facilitates the identification of different races, since the differential cultivars are assigned a binary value in the same fixed order (Table 2). Designation of races is facilitated by this system, since only odd numbers are assigned to races pathogenic on the most susceptible differential, cv. Michelite.

RESULTS

Isolates A, B, and X resembled more the morphology of the beta race when grown on PDA. Colonies appeared initially as white crystalline mycelia and became a dark gray after 10–12 days. These isolates did not sporulate on PDA, bean pod agar, or bean leaf-amended agar; sporulation was observed only after all three isolates were transfered to Mathur's agar. Colonies of isolate I, which resembled the morphology of the alpha race, developed black mycelia in 6 days, with abundant sporulation on all four media and particularly on PDA.

Table 1 gives the reactions of the four unknown isolates on the eight differential bean cultivars and six commercial check cultivars. The A, B, and X isolates produced similar reactions on all differential hosts, but reactions differed from those produced by the I isolate and by alpha and delta races. The A, B, and X isolates were pathogenic on cvs. Cornell 49-242 and Blackhawk, both of which carry the *Are* gene; on kidney bean cv. Isabella; and on pinto bean cvs. Aztec and U.I. 114. The kidney bean differential cv. Michigan Dark Red Kidney (DRK) was resistant, as were Montcalm DRK and navy bean differential cvs. Sanilac and Seafarer, both known to possess the *A* gene. The B isolate was pathogenic on Michelite (no. 1), Cornell 49-242 (no. 8), and Mexico 222 (no. 64) differentials and was categorized as race 73 based on the binary value (3). A variable response to isolates A, B, and X and the alpha race was observed on Mexico 222, indicating that genetic variability exists within the differential genotype.

The I isolate from Isabella attacked Michigan DRK, Coco a la Creme, and Sanilac differentials. The I isolate was also pathogenic on all *A* gene cultivars and nonpathogenic on *Are* gene cultivars (Table 1). With the exception of Coco a la Creme, this pattern of pathogenicity most resembled that of the delta race.

Table 1. Disease reactions[a] of four unknown isolates of *Colletotrichum lindemuthianum* on a series of eight differential bean cultivars[b] and six commercial cultivars compared with reactions of three known races of the pathogen

	Isolates				Known races		
Cultivars	I	B	X	A	Alpha	Delta	Alpha-Brazil
Differential							
Michigan DRK	S	R	R	R	R	S	R
Coco a la Creme	S	R	R	R	R	R	R
Sanilac	S	R	R	R	R	S	R
Kaboon	R	R	R	R	R	R	R
Cornell 49-242	R	S	S	S	R	R	S
PI 207262	R	R	R	R	R	R	R
Evolutie	R	R	R	R	R	R	R
Mexico 222	R	S	S	S	S	R	S
Commercial							
Blackhawk	R	S	S	S	R	R	...
Isabella	S	S	S	S	R	S	...
Aztec	S	S	S	S	R	S	...
Montcalm	S	R	R	R	R	S	...
Seafarer	S	R	R	R	R	S	...
Pinto U.I. 114	S	S	S	S	S	S	...

[a]S = susceptible, R = resistant. Reactions to alpha-Brazil race based on Drijfhout and Davis (4), Fouilloux (5), and Tu (16).
[b]Proposed by Drijfhout and Davis (4).

Table 2. Disease reactions[a] of two unknown isolates of *Colletotrichum lindemuthianum* on a series of 12 differential bean cultivars[b] compared with reactions of three known races of the pathogen

		Isolates		Known races		
Cultivars	Binary value[c]	I	B	Alpha	Delta	Alpha-Brazil
Michelite	1	S	S	R	S	S
Michigan DRK	2	S	R	R	S	R
Perry Marrow	4	S	R	R	S	R
Cornell 49-242	8	R	S	R	R	S
Widusa	16	R	R	R	S	S
Kaboon	32	R	R	R	R	R
Mexico 222	64	R	S	S	R	S
PI 207262	128	R	R	R	R	R
TO	256	R	R	R	R	R
TU	512	R	R	R	R	R
AB 136	1024	R	R	R	R	R
G 2333	2048	R	R	R	R	R

[a]S = susceptible, R = resistant. Reactions to alpha-Brazil race based on Drijfhout and Davis (4), Fouilloux (5), and Tu (16).
[b]Proposed by CIAT (3).
[c]Binary value is assigned to differential cultivars in the same fixed order as shown. Races are distinguished numerically by the sum of binary values assigned to those differential cultivars on which the unknown race is pathogenic (3).

On the CIAT differentials, isolate I attacked Michelite (no. 1), Michigan DRK (no. 2), and Perry Marrow (no. 4) and was categorized as race 7. These data suggest less similarity with the delta race (Table 2).

DISCUSSION

Two races of *C. lindemuthianum* previously not reported in the United States were identified on field-grown beans from Michigan and North Dakota in 1993. Isolates B and X, which came from the same location in Michigan, were similar to isolate A from North Dakota. The latter was found in North Dakota in 1992 on plants of cv. Aztec pinto bean grown from seeds produced in Michigan in 1991. Anthracnose was reported only once in North Dakota, but the isolate was not characterized (17). Aztec from the same seed source was grown in Ontario in 1992, where anthracnose symptoms also were observed. This was characterized as the alpha-Brazil race by Tu (16). The Ontario isolate and the A, B, and X isolates in the United States are similar in their ability to overcome the *Are* gene. However, none of the U.S. isolates tested were pathogenic on the Widusa differential, whereas the Ontario isolate (16) produced a susceptible reaction (grade 4 = 30–40%). In addition, the delta race has produced a mixed reaction on Widusa and the alpha race used in the study was not pathogenic on Widusa, which conflicts with literature reports (5). The observed differences could be the result of lack of genetic purity of the Widusa cultivar. It would appear that the original source of the A, B, X, and Ontario isolates of *C. lindemuthianum* was from Aztec seeds produced in 1991 on the MSU Research Farm in East Lansing.

Based on the CIAT binary values for the 12 differential cultivars (3), *C. lindemuthianum* isolates A, B, and X would be categorized as race 73. Apparently, race 73 of *C. lindemuthianum* now exists in North America, which poses a serious threat to many dry bean cultivars currently in production. Its ability to overcome the *Are* gene is very serious in Ontario, where the gene is extensively utilized in current cultivars. The importance of the *A* gene, which conditions resistance to race 73, should not be overlooked by breeders.

The I isolate, classified as race 7 using the same binary system, most resembled the delta race in its ability to overcome the *A* gene. This race has not been previously reported in the United States. The origin of this race is still under investigation, since it was identified in kidney bean germ plasm not previously observed to be infected with anthracnose. Although the delta race was first reported in Ontario in 1976, the disease has been effectively controlled through strict crop rotation, seed inspection, and seed treatment programs (15). The long-term strategy of incorporating the *Are* gene to control the delta race could be in jeopardy due to the appearance of race 73 or the alpha-Brazil race. The major concern in the United States is the lack of resistance afforded by the *A* gene to race 7, since the majority of dry bean cultivars carry only this single resistance gene.

Given the occurrence of two new races of *C. lindemuthianum* in North America, the most effective disease control strategy would be the pyramiding of the *A* and *Are* resistance genes into new cultivars. The *A*/*Are* combination would condition resistance to all known North American isolates of *C. lindemuthianum*, including races 7, 73, alpha-Brazil, and delta. Conventional procedures for gene pyramiding are not practical for plant breeders because of epistatic interactions between resistance genes. Since pyramiding requires that both epistatic and hypostatic resistance genes be combined in a single genotype, breeders have no convenient way to select for the hypostatic gene without using multiple inoculations with different races of a pathogen or test crossing back to a susceptible genotype. The process of pyramiding genes is made more efficient through marker assisted selection (MAS), if molecular markers linked tightly to the resistance genes were available, since the expression of the molecular marker is not masked by epistatic interactions. MAS has been demonstrated in breeding for resistance to bean common mosaic virus (BCMV) in common bean, where the *I*/*bc-3* gene combination affords pyramided resistance to all known strains of BCMV (7). Currently, a random amplified polymorphic DNA (RAPD) marker linked tightly (2.0 cM) to the *Are* gene has been identified (18), but another marker linked to the *A* gene needs to be identified. Since only a few dry bean cultivars possess the desirable *A*/*Are* gene combination, a group of 115 bean genotypes was inoculated with the B and I isolates to determine the genetic resistance within MSU germ plasm. Based on the reaction of these genotypes, race 73 was more pathogenic on small-seeded Mesoamerican beans, while race 7 was more pathogenic on the large-seeded Andean kidney beans (*data not shown*). Finally, in recognition of the serious nature of the pathogenic variability of *C. lindemuthianum*, a survey is under way to access the extent to which these new races are present both in bean breeding material at MSU and in commercial bean cultivars in Michigan. To date, the problem appears to be restricted to seed of breeding materials produced at the MSU research farms. The origin or entry of these new races into Michigan is still unknown.

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